REMARKS

Reconsideration and allowance are respectfully requested.

The Examiner's courtesy in granting the interview of October 18, 2001 is gratefully acknowledged. During the interview, he stated that he would rejoin method claims restricted to antigens that induce an immune response against a pathogen. Applicants have agreed to provide the references missing from the Examiner's application files so the Information Disclosure Statement submitted on July 20, 2001 could be considered.

Claims 1-25, 27-51 and 60-91 are pending. Applicants have canceled the non-elected claims without prejudice to future prosecution of that subject matter.

The amendments are supported by the original disclosure and, thus, no new matter has been added. If the Examiner should disagree, however, he is respectfully requested to point out the challenged limitation with particularity in the next Action so support may be cited in response.

The title has been amended to conform to the scope of the claims (see generic description of the invention in the Abstract of the Disclosure) and the specification has been amended to add sequence identifiers.

The claims have been amended to conform to the Examiner's restriction requirement as he stated it during the interview (i.e., rejoinder of method claims which are restricted to antigens that induce an immune response against a pathogen). They have also been broadened by removing the limitation to therapeutic effects (see definition of "effective amount" and the types of treatments which may be provided by induction of an immune response starting on page 9, line 32, of the specification). Adjuvants are broadly defined (see "Adjuvant" section starting on page 26, line 16, of the specification).

Claim amendments also clarify that pretreating disrupts at least the skin's stratum corneum but does not penetrate the skin's dermis (see page 5, lines 9-11, and page 46, lines 21-26, of the specification). Penetration enhancement affects the stratum corneum and possibly the superficial dermis, as well as possibly the intervening skin layer of the epidermis (see page 12, lines 27-29, of the specification). Claim 79 adds the further limitation that at least the skin's epidermis is disrupted.

Support for claim 2 is found inter alia on pages 11-12 and page 14, lines 1-20, of the specification. The addition of "propellant gun" to claim 18 is supported by page 12, lines 13-14, of the specification. Adjuvants are generically defined (see page 9, lines 30-31, of the specification) and include lipid A (page 26, line 32, of the specification) and CpG (page 27, line 7, of the specification).

Amended claims 23-30 and new claims 87-91 are directed to separating antigen and adjuvant components of the formulation to apply one to the pretreated area and to administer the other separately (see page 8, lines 1-16, of the specification).

Amended claims 29-30 and new claims 87-88 are directed to different routes of administration (see page 23, lines 20-22; page 61, lines 3-5; page 63, lines 26-28, of the specification). With respect to the physical state of the formulation, amended claim 50 includes a cream and new claim 86 is limited to a solution in accordance with page 14, line 28, of the specification. New claims 80-81 specify different disrupting devices listed in original claim 19. For new claim 82, hydration is described on page 11, lines 3-30, of the specification and its timing is illustrated in the Examples (e.g., pages 38, 41, 44, 46 and 48).

Claims 34-36 recite different pathogens from which the antigen may be derived (see page 8, lines 26-28, of the specification). Such antigens may be recombinantly produced (new claim 73), chemically synthesized (new claim 74), biochemically purified (new claim 75), or expressed by a whole organism (new claim 83) in accordance with page 9, lines 1-4 and 26-29, of the specification. Amended claim 38 is supported by page 9, lines 8-9, and page 24, line 5, of the specification. New claims 67-72 recite the molecular weight limitations described on page 8, lines 31-33, of the specification.

Similarly, adjuvants may be recombinantly produced (new claim 76), chemically synthesized (new claim 77), or biochemically purified (new claim 78) in accordance with page 9, lines 24-32; page 26, lines 23-24; and page 28, line 32, to page 29, line 4, of the specification. ADP-ribosylating exotoxins and derivatives thereof as adjuvants (amended claims 43-49 and new claims 60-63) are described on page 27, line 26, to page 29, line 21, of the specification. New claim 64 is supported by page 8, lines 30-31, of the specification. New claims 65-66 are complementary for the concept that a single

molecule may contain both antigen and adjuvant properties, or not (see page 26, lines 17-18, of the specification).

New claims 84-85 describe specific types of immune responses induced by the invention (page 105, line 3, of the specification).

Paper and computer readable forms of the Sequence Listing are being submitted herewith in response to the Examiner's requirement. The paper and computer readable forms of the Sequence Listing do not add new matter, and their contents are the same. It is respectfully submitted that this submission complies with 37 CFR § 1.821 et seq. Otherwise, prompt notice of any defects in the Sequence Listing is earnestly solicited and additional time is requested to comply.

A new declaration listing the parent application relied upon for priority is being submitted herewith along with a change of address to Nixon & Vanderhye. This patent application is assigned to the Government of the United States as represented by the Secretary of the Army The assignment is being submitted herewith for recordation.

35 U.S.C. 112 – Enablement

The Patent Office has the initial burden to question the enablement provided for the claimed invention. M.P.E.P. § 2164.04, and the cases cited therein. It is incumbent upon the Patent Office, whenever a rejection on this basis is made, to explain why it doubts the truth or accuracy of any statement in a supporting disclosure and to back up assertions of its own with acceptable evidence or reasoning which is inconsistent with the contested statement. *In re Marzocchi*, 169 USPQ 367, 370 (C.C.P.A. 1971). Specific technical reasons are always required. See M.P.E.P. § 2164.04.

Claims 1-7, 21, 23-37, 39-43, 45, 50-51, 55 and 57 were rejected under Section 112, first paragraph, because the specification allegedly "does not reasonably provide enablement for, a method of inducing an *enhanced therapeutically effective* immune response, said method comprising pretreating an area of skin an applying an antigen with an *adjuvant*." Applicants traverse.

As an initial matter, Applicants note that the enhancement which is the subject of the claims refers to enhancing skin penetration by a formulation. This may be caused by chemical or physical penetration enhancers or disruption devices. Enhanced skin

penetration by the formulation induces an immune response which method may be used to treat an organism "to treat existing disease, protectively to prevent disease, or to reduce the severity and/or duration of disease" (page 7, lines 16-25, and page 10, lines 1-4, of the specification).

As stated in the Office Action, the Examples show that the invention (i.e., transcutaneous immunization with penetration enhancement) is able to induce an immune response. But on page 3 of the Action, it was asserted, "It is well known in the immunological arts that an immune response can be generated that is not therapeutically effective." The three references cited in support of this assertion do not, however, utilize transcutaneous immunization so their relevance to the claimed invention has not been established. Such references do not satisfy the Patent Office's burden under *Marzocchi* to provide "acceptable evidence or reasoning which is inconsistent with the contested statement" because they do not provide "specific technical reasons" to contradict Applicants' teaching that the claimed invention is capable of therapeutic application. Moreover, it would not require undue experimentation to determine whether the immune response that is induced was sufficient to be therapeutically effective.

In contrast, Applicants have cited Glenn et al. (J. Immunol., 161, 23211-3214, 1998) on page 89 of the specification and incorporated its contents by reference. The reference shows that transcutaneous immunization without penetration enhancement using cholera toxin will protect against a lethal toxin challenge (cf. protection of vaccinated organism against "toxin secretion" as described on page 7, lines 18-19 and 24, of the specification). Although such a lethal toxin challenge was not performed after transcutaneous immunization with penetration enhancement in this application, there is no reason to believe that the therapeutic efficacy of transcutaneous immunization would be reduced by use of chemical or physical penetration enhancers or disruption devices.

Thus, there is no relevant evidence of record that is inconsistent with statements in Applicants' specification that the induced immune response is capable of therapeutic application. Furthermore, Glenn et al. (1998) is evidence in support of the therapeutic efficacy of transcutaneous immunization even without penetration enhancement!

On page 3 of the Action, it was further asserted that "Examples 7 and 10 clearly disclose that [diphtheria toxin] will not function as an adjuvant in the claimed method."

This is a mischaracterization of what was being shown in Applicants' Examples. The DT being used in Examples 7 and 10 is diphtheria toxoid (page 50, line 17, and page 57, line 10, of the specification), not diphtheria toxin. This particular toxoid derivative of diphtheria toxin does not have adjuvant activity; it was being used only as an antigen. This was an important demonstration because it showed that cholera toxin acted as an adjuvant by inducing a DT-specific immune response whereas diphtheria toxoid alone did not induce a DT-specific immune response (see page 9, lines 30-31, of the specification). Therefore, cholera toxin is able to act as an ADP-ribosylating exotoxin having adjuvant activity that is effective in inducing an immune response to a co-administered antigen like diphtheria toxoid.

Thus, Examples using diphtheria toxoid (DT) do not support the assertion that "the invention as broadly claimed must be considered highly unpredictable" because this particular toxoid lacks adjuvant activity and is being used only as an antigen. It is taught in the specification that the diphtheria toxoid used in the Examples lacks adjuvant activity, whereas other ADP-ribosylating exotoxin derivatives (e.g., B subunits) have adjuvant activity. Claim 43 has been amended to clarify that the derivative has such adjuvant activity, although it should be noted that the B subunit alone possesses both antigen and adjuvant activities.

Withdrawal of the Section 112, first paragraph, rejection is requested because it would not require undue experimentation for a person of skill in the art to make and use the claimed invention.

Double Patenting

Claims 1-7, 21, 23-37, 39-43, 45, 50-51, 55 and 57 were provisionally rejected under the judicially-created doctrine of obviousness-type double patenting over claims 1-12, 16-24 and 27-35 of copending U.S. Appln. 09/266,803 and claims 1-8, 11-23 and 29-30 of copending U.S. Appln. 09/316,069. Applicants traverse because a terminal disclaimer is being submitted.

It should be noted that filing of a terminal disclaimer to overcome a rejection based on nonstatutory double patenting is not an admission that the rejection was proper. See *Quad Environmental Technologies Corp. v. Union Sanitary District*, 20

USPQ2d 1392, 1394-95 (Fed. Cir. 1991). The Court stated that the "filing of a terminal disclaimer simply serves the statutory function of removing the rejection of double patenting, and raises neither a presumption nor estoppel on the merits of the rejection." Thus, submission of a terminal disclaimer in accordance with M.P.E.P. § 804.02 is not an admission that the pending claims are obvious over the claims of the cited copending applications.

Withdrawal of the double patenting rejection is requested.

Conclusion

Having fully responded to all of the pending objection and rejections of the Office Action (Paper No. 15), Applicants submit that the claims are in condition for allowance and earnestly solicit an early Notice to that effect. The Examiner is invited to contact the undersigned if any further information is required.

Respectfully submitted,

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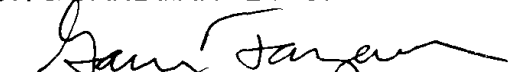
By: 

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APPENDIX

MARKED-UP VERSION TO SHOW CHANGES

IN THE TITLE

The title is amended as follows: USE OF PENETRATION ENHANCERS AND BARRIER DISRUPTION AGENTS TO ENHANCE THE TRANSCUTANEOUS IMMUNE RESPONSE [INDUCED BY ADP-RIBOSYLATING EXOTOXIN]

IN THE SPECIFICATION

The specification is amended as follows.

Page 50, third paragraph starting on line 24:

Co-administration of DT and a control DNA sequence (SEQ ID NO:1; CpG2: *TCCAATGAGCTTCCTGAGTCT*) failed to induce a detectable rise in the anti-DT titers. In contrast, addition of a DNA sequence containing an unmethylated CpG dinucleotide flanked by two 5' purines and two 3' pyrimidines (SEQ ID NO:2; [CpG1 (immunostimulatory DNA)] immunostimulatory CpG1: *TCCATGA***CG***TTCCTGACGTT*) resulted in a detectable increase in the serum anti-DT IgG titer in 5 of 5 animals. Thus it appears that bacterial DNA containing appropriate motifs such as CPGs (6KD) can be used as adjuvant to enhance delivery of antigen through the skin for induction of antigen specific antibody responses.

Page 51, first paragraph starting on line 5:

The transcutaneous effect of transcutaneous immunization can also be detected by T-cell proliferation. BALB/c mice 6 to 8 weeks of age were shaved and anesthetized as described above for the "immunization procedure". On the day of immunization the backs of the mice were wiped with isopropanol. After the alcohol had evaporated (approximately 5 minutes), 100 µl of phosphate buffered saline (PBS) containing 100 µg of DNA (CpG1 or CpG2) and 100 µg of diphtheria toxoid (DT) was applied to the back for 90 to 120 minutes. Oligonucleotides were synthesized by Oligos Etc with a phosphorothioate backbone to improve stability. Removal of excess antigen was

conducted as described in the "immunization procedure." The immunization was repeated 4 and 8 weeks later. Twelve weeks after the primary immunization draining (inguinal) LNs were removed and pooled from five immunized animals. The capacity to proliferate in response to media or antigen (DT) was assessed in a standard 4 day proliferation assay using 3-H incorporation as a readout. The results are shown in Table 7B. Co-administration of DT and a DNA sequence containing an unmethylated CpG dinucleotide flanked by two 5' purines and two 3' pyrimidines (SEQ ID NO:2 [CpG1 (immunostimulatory DNA): *TCCATGA**CG**TTCCTGACGTT*]) resulted in a detectable increase in the antigen specific proliferative response. Thus it appears that bacterial DNA containing appropriate motifs can be used as adjuvant to enhance delivery of antigen through the skin for induction of proliferative responses.

Page 59, third paragraph starting on line 22:

Co-administration of SLA and CpG1 (immunostimulatory DNA containing an unmethylated CpG dinucleotide flanked by two 5' purines and two 3' pyrimidines – SEQ ID NO:2 [*TCCATGA**CG**TTCCTGACGTT*]) or CT resulted in a detectable increase in the antigen specific proliferative response. However, the antigen (SLA) specific proliferative response was approximately 20 times higher in lymph node cell cultures from animals exposed simultaneously to both CpG1 and CT as compared to cultures derived from animals exposed to either adjuvant alone. Thus it appears that bacterial DNA containing appropriate motifs synergizes with ADP ribosylating exotoxins such as CT as adjuvants on the skin to induce higher immune responses than[t] to either adjuvant alone.

IN THE CLAIMS

The claims are amended as follows.

1. (Amended) A method for inducing an [enhanced therapeutically effective] immune response in a[n] subject comprising:

a. pretreating an area of [the] skin of said subject, whereby said pretreating disrupts at least the skin's stratum corneum but does not penetrate the skin's dermis; and

b. applying [to said pretreated area] a formulation to said pretreated area, wherein said formulation comprises [comprising]:

1) [a therapeutically effective amount of] at least one antigen sufficient to induce an immune response against a pathogen,

2) at least one adjuvant present in an amount effective to induce said [promote an] immune response to said at least one antigen, and

3) a pharmaceutically acceptable carrier [to the skin of said subject], wherein said pretreating enhances skin penetration by said formulation [pretreated area is not perforated].

2. (Amended) The method of claim 1, wherein said pretreating comprises applying a chemical to said area of skin to enhance[s] skin penetration by said formulation.

4. (Amended) The method of claim 3, wherein said patch is selected from the group consisting of an occlusive dressing, a[n] nonocclusive dressing, a hydrogel dressing and a reservoir dressing.

7. (Amended) The method of claim 5, wherein said swab contains [is treated with] an alcohol or a composition containing alcohol.

8. (Amended) The method of claim 5, wherein said swab contains [is treated with] acetone or a composition containing acetone.

10. (Amended) The method of claim 5, wherein said swab contains [is treated with] a detergent or a detergent solution.

18. (Amended) The method of claim 1, wherein said pretreating comprises disrupting the surface layer of said pretreated area with a disrupting device or propellant gun.

20. (Amended) The method of claim 1, wherein said adjuvant is at least one of the members selected from the group consisting of bacterial DNA, CpG, cytokines, chemokines, tumor necrosis factor alpha, genetically altered toxins, chemically conjugated toxins, lipid A and lipopolysaccharides.

21. (Amended) The method of claim 20, wherein said [therapeutically effective] immune response results in LN cell proliferation.

22. (Amended) The method of claim 20 wherein said adjuvant is a combination of at least two of the adjuvants selected from the group consisting of bacterial DNA, CpG, cytokines, chemokines, tumor necrosis factor alpha, genetically altered toxins, chemically conjugated toxins, lipid A and lipopolysaccharides.

23. (Amended) A method for inducing an [enhanced therapeutically effective] immune response in a[n] subject comprising:

a. pretreating an area of skin of said subject, whereby said pretreating disrupts at least the skin's stratum corneum but not does not penetrate the skin's dermis;

b [a]. applying [to said pretreated area] an adjuvant formulation to said pretreated area, wherein said adjuvant formulation comprises [comprising]:

1) [a therapeutically effective amount of at least one antigen,

2)] at least one adjuvant present in an amount effective to promote said [an] immune response [to the antigen,] and

2 [3]) a pharmaceutically acceptable carrier [to the skin of said subject], wherein said pretreating enhances skin penetration by said adjuvant formulation [pretreated area is not perforation]; and

c [b]. administering to said subject a separate antigen formulation comprising at least one antigen sufficient to induce an immune response against a pathogen [to said subject].

24. (Amended) The method of claim 23, wherein said separate antigen formulation is administered to said subject at a time after said applying of said adjuvant

formulation to said pretreated area[, wherein said separate antigen formulation provides a further immune response in said subject].

25. (Amended) The method of claim 23, wherein said separate antigen formulation is administered to said subject at a time before said applying of said adjuvant formulation to said pretreated area[, wherein said separate antigen formulation provides a further immune response in said subject].

27. (Amended) The method of claim 23 [26], wherein said separate antigen formulation is administered to said subject about simultaneously with said applying of said adjuvant formulation to said pretreated area [application of said antigen and said adjuvant occur about simultaneously].

28. (Amended) A method for inducing an [enhanced therapeutically effective] immune response in a[n] subject comprising:

a pretreating an area of the skin of said subject, whereby said pretreating disrupts at least the skin's stratum corneum but does not penetrate the skin's dermis;

b applying an antigen formulation to said pretreated area, wherein said antigen formulation comprises:

1) at least one antigen sufficient to induce an immune response against a pathogen and

2) a pharmaceutically acceptable carrier, wherein said pretreating enhances skin penetration by said antigen formulation; and

c administering to said subject a separate adjuvant formulation comprising at least one adjuvant in an amount effective to promote said immune response [administering an effective amount of at least one antigen; and administering an effective amount of at least one adjuvant, wherein at least one of said antigen and said adjuvant are administered on said pretreated area and said pretreated area is not perforated].

29. (Amended) The method of claim 28, wherein [said antigen is administered to said pretreated area and] said separate adjuvant formulation is administered by intramuscular injection or a route [process] selected from the group consisting of [intramuscular injection,] oral, buccal, nasal, [and] rectal, vaginal and intradermal.

30. (Amended) The method of claim 28, wherein said separate adjuvant formulation is parenterally administered [to said pretreated area and said antigen is administered by a process selected from the group consisting of intramuscular injection, oral, nasal and rectal].

31. (Amended) The method of claim 1, wherein said antigen presents on a cell surface of a Langerhans cell to a lymphocyte, thereby inducing the immune response in the subject [organism].

32. (Amended) The method of claim 31, wherein exposure to said adjuvant causes migration of the Langerhans cell to a lymph node.

33. (Amended) The method of claim 31, wherein exposure to said adjuvant signals the Langerhans cell to mature into a dendritic cell.

34. (Amended) The method of claim 1, wherein the antigen is derived from a bacterium [source selected from the group consisting of a pathogen, a tumor cell and a normal cell].

35. (Amended) The method of claim 1, wherein the antigen is derived from a [pathogen selected from the group consisting of a bacteria,] virus[, fungus and parasite].

36. (Amended) The method of claim 1, wherein the antigen is derived from a fungus or a parasite or [a selected from the group consisting of a tumor antigen, an autoantigen, an allergen and] a biological warfare agent.

38. (Amended) The method of claim 1, wherein the formulation further comprises a live or an attenuated live virus or a virosome, and the at least one antigen is expressed by the live or attenuated live virus or virosome.

43. (Amended) The method of claim 1, wherein the adjuvant comprises at least one [is an] ADP-ribosylating exotoxin or a derivative thereof having adjuvant activity.

44. (Amended) The method of claim 43, wherein the ADP-ribosylating exotoxin [adjuvant] is cholera toxin (CT) [or cholera toxin B subunit (CTB)].

45. (Amended) The method of claim 43, wherein the ADP-ribosylating exotoxin [adjuvant] is *E. coli* heat-labile enterotoxin (LT) [or pertussis toxin].

46. (Amended) The method of claim 43, wherein the [adjuvant in said formulation is provided as a nucleic acid encoding an] ADP-ribosylating exotoxin is pertussis toxin (PT).

47. (Amended) The method of claim 43 [1], wherein the ADP-ribosylating exotoxin is diphtheria toxin (DT) [antigen in the formulation is provided as a nucleic acid including a sequence encoding the antigen].

48. (Amended) The method of claim 1 [47], wherein the formulation comprises a mutant ADP-ribosylating exotoxin [nucleic acid is non-integrating and non-infectious].

49. (Amended) The method of claim 1 [47], wherein the formulation comprises an ADP-ribosylating exotoxin B subunit [nucleic acid further includes a regulatory region operably linked to the sequence encoding the antigen].

50. (Amended) The method of claim 1, wherein the formulation is a cream or gel or emulsion or ointment.

Claims 26 and 52-59 are canceled without prejudice or disclaimer.

Claims 60-91 are added as new claims.

IN THE SEQUENCE LISTING

Paper and computer readable copies of the Sequence Listing are attached.